Light-Induced Nanosecond Relaxation Dynamics of Rhenium-Labelled *Pseudomonas aeruginosa* Azurins

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SUPPORTING INFORMATION

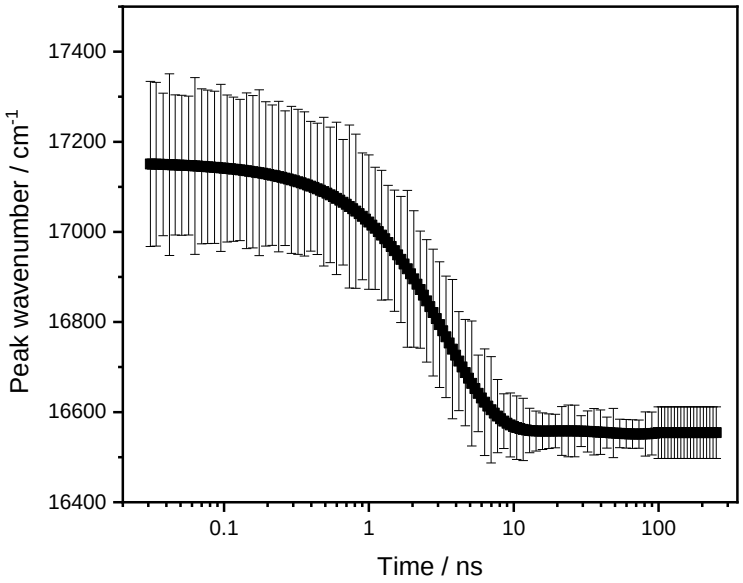

Figure S1. Typical time-dependence of luminescence band-maximum wavenumbers of Re-azurins including experimental errors determined by the bootstrap method. (The case of Re(phen)124K122.)

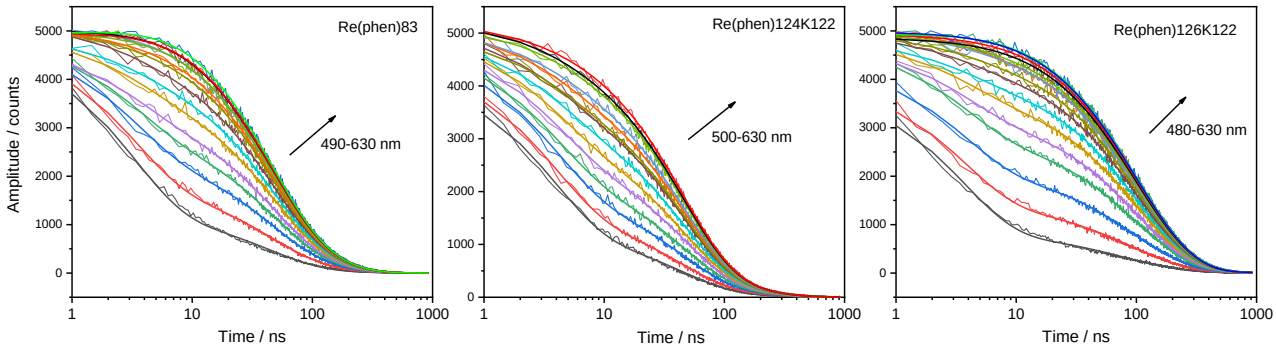

Figure S2. Luminescence decay profiles of selected Re(phen)-azurins measured at different emission wavelengths in 10 nm intervals. Raw experimental data are shown together with their global fits.

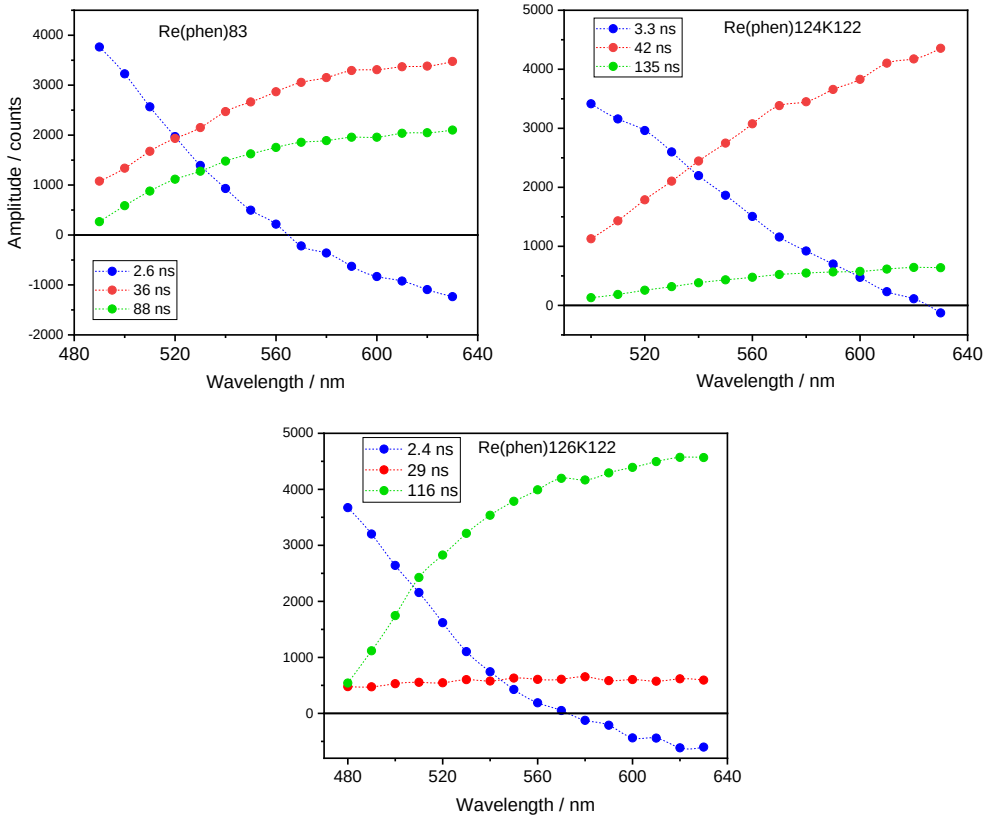

Figure S3. Luminescence decay lifetimes of Re(phen)-azurins and wavelength-dependences of the corresponding amplitudes (i.e., decay associated spectra). Obtained by 3-exponential global fitting of intensity decays shown in Figure S2. Lifetime accuracy ±2% or better. Amplitude accuracy 5% or better, larger errors occur for the blue data (red for Re(dmp)83) at and around switching from positive to negative values, indicating that 2-exponential fits would be sufficient in these regions

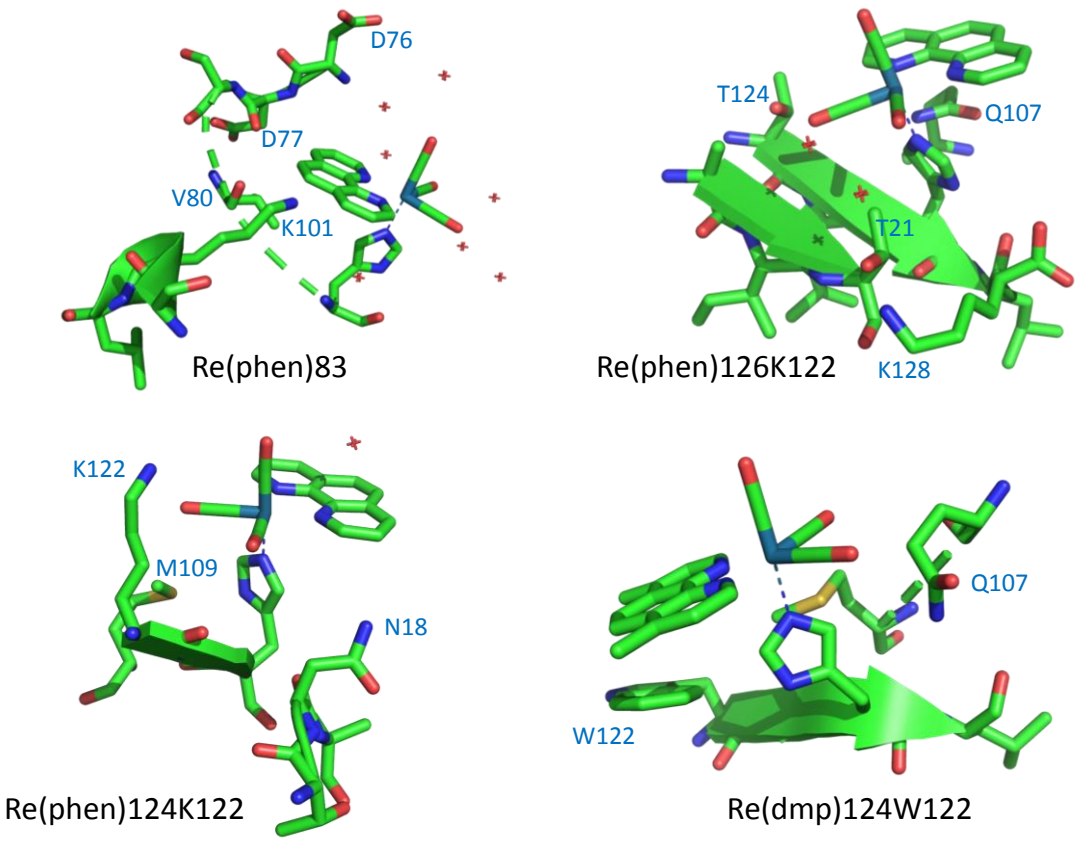

Figure S4. Rhenium binding sites of investigated Re-azurins. Taken from experimental crystal structures.[1,2,3,4] (Closest distances reported below.)

Re(phen)83 (1JZI): H83 in a flexible/dynamic loop. D76 and D77 residues of the same loop surround the binding site (D77 backbone C=O – phen distance 3.7 Å). Nitrogen atom of the K101 side chain 4.3 Å from phen. V80 4.2 Å from phen. C≡O ligands are asymmetrically solvated by H_2O molecules in the range 3.1 – 3.7 Å (Two H_2O per CO. O-atoms shown as red crosses.).

Re(phen)126K122 (3IBO): H126 at the end of a rigid β-sheet. The T124 (of the same β-sheet) -OH group 3.0 Å from an equatorial C≡O, H-bonding possible. Q107 (part of a loop) side chain 3.3-3.5 Å below phen, 3.9 Å from H126-imidazole. T21 (part of a β-sheet) 3.6 Å apart from H126-imidazole. Flexible side chain of the terminal K128 occurs 4.9 Å from H126 imidazole and 6.2 Å from equatorial C≡O. The Re chromophore is exposed to solvent but the degree of solvation could be affected by dimerization.[5,6]

Re(phen)124K122 (2I7S): H124 in a rigid β-sheet. Polar K122 side chain 4.3 – 5 Å from the axial C≡O. Amide (NH_2) of N18 (part of a β-sheet) 3.9 Å from phen and 4.6 Å from one equatorial C≡O. H124 close to a flexible M109 side chain. The Re chromophore is exposed to solvent but the degree of solvation could be affected by dimerization.[5,6]

Re(dmp)124W122 (2I7O): H124 in a rigid β-sheet. W122-indole (the same β-sheet) is in a π-π interaction with dmp (3.4 Å, angle 20.9°). Q107 (part of a loop) 3.5 Å from an equatorial C≡O (H-bonding possible). H124 close to a flexible M109 side chain. The Re chromophore is exposed to solvent but the degree of solvation could be affected by dimerization.[5,6]

Table S1. Results of 3-exponential fits of individual luminescence decay profiles measured at selected emission wavelengths in the blue, near-maximum, and red parts of the emission bands.

Re-azurin	Wavelength nm	τ_1 (A_1) ns (counts)[a]	τ_2 (A_2) ns (counts)[a]	τ_3 (A_3) ns (counts)[a]
Re(dmp)83Zn	490	2.2 (2530) -	12.3 (1000) 5.6 (2560)[b]	662 (2120) 647 (2130)[b]
	560	2.0 (230) -	4.4 (1050) 4.1 (1230)[b]	680 (4040) 681 (4040)[b]
	650	3.6 (630)	33 (-480)	680 (5030)
Re(dmp)83	470	1.8 (5150)	22 (1230)	178 (268)
	560	17 (680) -	50 (3370) 43 (3790)	465 (980) 360 (1070)
	630	2.4 (-30)	50 (3830)	420 (1190)
Re(dmp)124K122	470	3.0 (2760)	16 (1500)	369 (1100)
	560	2.7 (260) -	85 (800) 73 (750)[b]	431 (3850) 419 (3900)[b]
	690	4.7 (-1730)	150 (1520)	506 (3640)
	700	4.9 (-1840)	134 (1250)	460 (3922)
Re(dmp)124W122	470	2.1 (2690)	12.8 (2090)	72 (670)
	560	4.7 (1730)	41 (2270)	106 (1270)
	690	1.5 (1110)	37 (1834)	98 (1760)

[a] Data acquisition stopped at 5000 counts. [b] Statistically equivalent 2-exponential fit.

References

1. Crane, B. R.; Di Bilio, A. J.; Winkler, J. R.; Gray, H. B., Electron Transfer in Single Crystals of Pseudomonas aeruginosa Azurins. *J. Am. Chem. Soc.* **2001,** *123*, 11623-11631.

2. Blanco-Rodríguez, A. M.; Busby, M.; Grădinaru, C.; Crane, B. R.; Di Bilio, A. J.; Matousek, P.; Towrie, M.; Leigh, B. S.; Richards, J. H.; Vlček, A., Jr.; Gray, H. B., Excited-State Dynamics of Structurally Characterized $[Re^{I}(CO)_3(phen)(HisX)]^+$ (X = 83, 109) Pseudomonas aeruginosa Azurins in Aqueous Solution. *J. Am. Chem. Soc.* **2006,** *128*, 4365-4370.

3. Blanco-Rodríguez, A. M.; Busby, M.; Ronayne, K. L.; Towrie, M.; Grădinaru, C.; Sudhamsu, J.; Sýkora, J.; Hof, M.; Záliš, S.; Di Bilio, A. J.; Crane, B. R.; Gray, H. B.; Vlček, A., Jr., Relaxation Dynamics of $[Re^{I}(CO)_3(phen)(HisX)]^+$ (X = 83, 107, 109, 124, 126) *Pseudomonas aeruginosa* Azurins. *J. Am. Chem. Soc.* **2009,** *131*, 11788-11800.

4. Shih, C.; Museth, A. K.; Abrahamsson, M.; Blanco-Rodriguez, A. M.; Di Bilio, A. J.; Sudhamsu, J.; Crane, B. R.; Ronayne, K. L.; Towrie, M.; Vlček, A., Jr.; Richards, J. H.; Winkler, J. R.; Gray, H. B., Tryptophan-Accelerated Electron Flow Through Proteins. *Science* **2008,** *320*, 1760-1762.

5. Sokolová, L.; Williamson, H.; Sýkora, J.; Hof, M.; Gray, H. B.; Brutschy, B.; Vlček, A., Jr. , Mass Spectrometric Characterization of Oligomers in *Pseudomonas aeruginosa* Azurin Solutions. *J. Phys. Chem. B* **2011,** *115*, 4790–4800.
6. Blanco-Rodríguez, A. M.; Di Bilio, A. J.; Shih, C.; Museth, A. K.; Clark, I. P.; Towrie, M.; Cannizzo, A.; Sudhamsu, J.; Crane, B. R.; Sýkora, J.; Winkler, J. R.; Gray, H. B.; Záliš, S.; Vlček, A., Jr., Phototriggering Electron Flow through Re^{I}-modified *Pseudomonas aeruginosa* Azurins. *Chem. Eur. J.* **2011,** *17*, 5350 – 5361.